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Peniophora pseudonuda is a synonym of *P. laeta*

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Abstract — *Peniophora laeta* is easily recognized because it is restricted to *Carpinus* as host in Europe, and the reddish yellow basidioma is provided with prominent teeth or hyphal pegs, disrupting the bark when developing. *P. pseudonuda* was earlier not even thought of as related to *P. laeta*, because basidiomata are smooth and developing on the bark. Moreover, basidioma initiation starts with a thin layer of brown-pigmented hyphae on the bark surface. This gives a bluish tint to the mature basidioma, which is in striking contrast to the orange-yellow basidiomata found in *P. laeta*. Nevertheless, both ITS sequences and crossing tests show that *P. pseudonuda* is conspecific with *P. laeta*. This was supported also by similarities in spores, basidia, and cystidia morphology.

Key words — *Corticiaceae*, epicortical basidiomata, spore morphometrics

Introduction

The corticioid fungus *Peniophora pseudonuda* was described in 1980, firstly as a species with restricted natural range, known from hyrcanian forests of northern Iran, in Elburz Mountains (Hallenberg 1980). Later it was collected and published from the northwestern part of Main Caucasus, in Krasnodar Province, Russia, in temperate broadleaved communities of *Quercus*, *Fagus*, and *Fagus-Abies* forest belts (Mukhamedshin 1992, Hallenberg et al. 1996). The species epithet reminds on the presence of wide broadly clavate gloeocystidia,

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similar to those in *P. nuda* (Fr.) Bres. The brown-pigmentation of hyphae in the subiculum was a reason why this taxon was referred to the subgenus *Peniophora* (Boidin 1994).

Peniophora laeta is a fungus distributed in Europe and Pacific part of North America (Ginns & Lefebvre 1993, Boidin 1994). Until 1957 *P. laeta* was not distinguished from *P. incarnata* s. l. (Donk 1957), and due to light-pigmented hyphae it has been referred to the subgenus *Gloeopeniophora*.

Materials and methods

Morphology

Specimens were studied in 5% potassium hydroxide (KOH), Melzer´s reagent (IKI) and Cotton Blue in lactic acid (CB). Measurements and drawings were made in KOH solution; spore measurements are based on at least thirty spores. In each range, the values in the parentheses are 10% of variation extremes.

Sampling and crossing tests

The specimens studied (Table 1) were selected from the FCUG culture collection (http://www.systbot.gu.se/database/FCUG/FCUG.html) at the University of Gothenburg (Sweden).

Crossing tests were restricted to specimens for which non-clamped single spore isolates were available. Single-spore mycelia from different specimens were placed in pairs on malt-extract agar (1.25% malt extract) and left in room temperature for three weeks. From each specimen, two to four single-spore mycelia were used. Paired cultures were checked for clamp formation in three different regions: at the immediate contact zone and on opposite sides of the inocula, some 20 mm from respective inoculum. Plates with negative results were re-checked after an additional three weeks.

DNA extraction, amplification, and sequencing

For crossing tests and as a source of DNA extraction, single-spore mycelium was isolated, cultivated on malt agar plates (1.25% malt extract), and subsequently placed in malt liquid solution (malt extract as above) for three weeks. When single-spore mycelium was not available, polyspore mycelium was used. Mycelia were harvested and dried between sheets of sterile filter paper; approximately 2 mg (dry-weight) of input mycelium were used per specimen. DNA extraction was accomplished using the DNeasy® Plant Mini Kit (QIAGEN®); during this and the following steps of the DNA preparation, purification, and sequencing, the recommendations of the respective manufacturer were followed.

The polymerase chain reactions were carried out using Ready-To-Go™ PCR Beads kits (Amersham Pharmacia Biotech), a Biometra TRIO-Thermoblock (Biometra, Germany), the PCR primers ITS1F and ITS4B, and the PCR set-up of Gardes & Bruns (1993). The PCR product was purified using QIAquick™ Spin procedure (QIAGEN®) and the sequence reactions were conducted using 100 ng of template DNA and the CEQ 2000 Dye Terminator Cycle Sequencing with Quick Start Kit (Beckman Coulter). Sequences were obtained using the CEQ 2000XL DNA Analysis System (Beckman Coulter).

Results and discussion

Molecular divergence and crossing tests

The ITS1 and ITS2 sequences were aligned manually and divergence was small. In total, the maximum variation between the samples in Table 1 were 1.9%, which is clearly within intraspecific variation (Nilsson et al. 2008). Moreover, crossing tests indicated conspecifity between the two species (Table 2).

Table 1. Details of the studied specimens. The substrate is specified to the extent known. The abbreviation 'dec.' refers to deciduous wood. FCUG numbers in bold were used for crossing tests.

Taxon / FCUG nr.	Locality	Substratum	Other number	GenBank
Peniophora laeta				
FCUG 1005	Romania, Iasi	*Carpinus*	NH 7998	GU322862
FCUG 1266	Sweden, Scania	*Carpinus*	NH 8557	GU322861
FCUG 1475	Romania, Cluj	dec. wood	NH 9358	GU322864
FCUG 1905	Sweden, Öland	*Carpinus*	EL 87-1	GU322860
FCUG 2729	Russia, Krasnodar	*Carpinus*	NH 13150	GU322863
Peniophora pseudonuda				
FCUG 86	Iran, Golestan	dec. wood	NH 2555	GU322867
FCUG 2384	Russia, Krasnodar	dec. wood	NH 12298	GU322866
FCUG 2390	Russia, Krasnodar	*Carpinus*	NH 12003	GU322865
FCUG 2664	Russia, Krasnodar	dec. wood	NH 12930	GU322868
FCUG 2681	Russia, Krasnodar	*Carpinus*	NH 12978	GU322869

Table 2. Results of crossing tests. All performed crossings resulted in clamp formation (+).

Taxon	Substratum	FCUG culture	1005	1266	1475	1905	2729	2384	2390
P. laeta	*Carpinus*	1005		+	+	+	+	+	+
P. laeta	*Carpinus*	1266			+	+	+	+	+
P. laeta	deciduous wood	1475				+	+	+	+
P. laeta	*Carpinus*	1905					+	+	+
P. laeta	*Carpinus*	2729						+	+
P. pseudonuda	deciduous wood	2384							+
P. pseudonuda	*Carpinus*	2390							
P. pseudonuda	deciduous wood	86						+	+

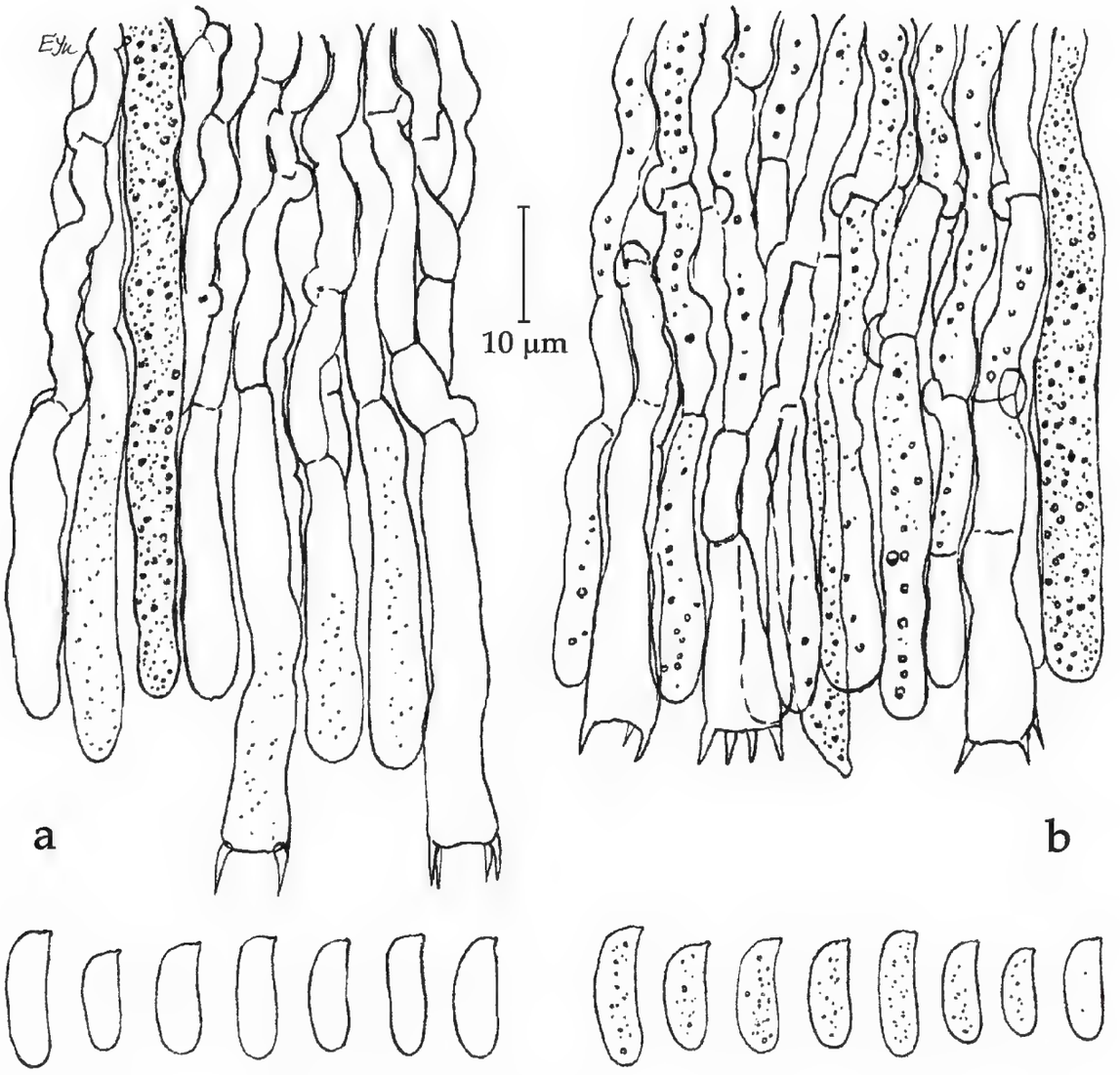

FIG. 1. Hymenium, subbasidial hyphae, and basidiospores in *Peniophora pseudonuda* (a, GB12298/FCUG 2384) and *P. laeta* (b, MSK 6943). Depending on the view, only 2 or 3 sterigmata of 4 are visible on basidia.

Macromorphologically, decorticating samples are well distinguished from non-decorticating: they have wart-like to hydnoid hymenophore projections, hymenophore color varies from pinkish or cream to light ochraceous. Basidiomata of *P. pseudonuda* always develop epicortically, hymenial surface is smooth, and the color varies from whitish cream with brownish hue to pale ochraceous and bluish grey. Thus, hymenium colors are partly overlapping in the two taxa.

On the other hand, the comparison of basidioma micromorphology of *P. pseudonuda* and *P. laeta* has shown a notable similarity in several characters. The shapes of spores and basidia are indistinguishable and hyphae are also very similar (FIG. 1). Morphometrics of the spores have demonstrated that there is

Table 3. Spore sizes in *Peniophora laeta* samples.

Basidioma growth habit*	Reference collection nr.	Region / latitude	Spore size range / arithmethical means (n=30), µm
d	FCUG 1266/ NH 8557	Sweden, Scania/ 56° N	(9.8–)10.6–12(–12.5) × (3.1–)3.3–4.2(–4.5)
d	MSK-F 6738	Belarus, Asipovichy / 53.3° N	7.5–11 × 2.7–4.1 / 8.79 × 3.31
d	MSK-F 7076	Belarus, Hlusk / 52.8° N	8–11.4 × 2.8–4.2 / 9.66 × 3.36
d	MSK-F 4560	Belarus, Petrykau / 52.2° N	8–11.5 × 2.2–3.7 8.87 × 3.08
d	KW 17598	Ukraine, Kyiv / 50° N	8.1–11.5 × 2.8–4.2 / 9.66 × 3.59
d	CWU(myc) Ch-24	Ukraine, Cherkasy / 49.7° N	7.6–11.2 × 2.2–4.1 / 9.21 × 3.05
d	KW 17590	Ukarine, Kirovhrad / 48.4° N	8.7–12.8 × 3–4.5 / 10.15 × 3.59
d	MSK-F 5981	Ukraine, Crimea / 45° N	7.5–11.7 × 2.5–4.1 / 9.28 × 3.29
nd	FCUG 2384/ NH12298	Russia, Krasnodar / 44° N	7.2–11.2 × 2.2–3.7 / 9.01 × 3.00
nd	MSK 6688	Russia, Stavropol' / 43.9° N	7.2–10.6 × 2.7–3.5 / 8.79 × 3.12
nd	Ghobad-Nejhad 413	Iran, E. Azerbaijan / 38.8° N	(8.3–)9–12(–13) × (3–)3.5–4.4(–5)
nd	FCUG 86/ NH2555	Iran, Golestan / 37.3° N	10–12(–13) × 4–5

* d – decorticating; nd – non-decorticating. The same abbreviations in Specimens examined.

no distinction that can be treated as specific (Table 3). Besides, variation in spore size does not display any dependence on geographical latitude.

Gloeocystidia are of variable morphology, depending on the age of basidioma and their position in certain parts of the basidioma. *P. pseudonuda* has numerous ellipsoid-clavate gloeocystidia, while *P. laeta* has predominantly subcylindrical ones, but all shapes of gloeocystidia which were observed in *P. pseudonuda*, were also found in *P. laeta* though in different frequency (Fig. 2, 3). Lamprocystidia are rare or scattered in both taxa, but usually more frequent in *P. pseudonuda*. The main micromorphological difference between them is the composition of subiculum. In *P. pseudonuda* there is a more or less pronounced basal layer, always of compact, agglutinated hyphae, while in *P. laeta* three different types of subicular layers can be recognized: (1) a more or less thin layer of compact subhorizontal hyphae (Fig. 3), (2) a much thicker layer of intertwined and loosely arranged hyphae (Fig. 4a), and (3) a layer of wide, short-celled hyphae, agglutinated and parallelly arranged, forming a

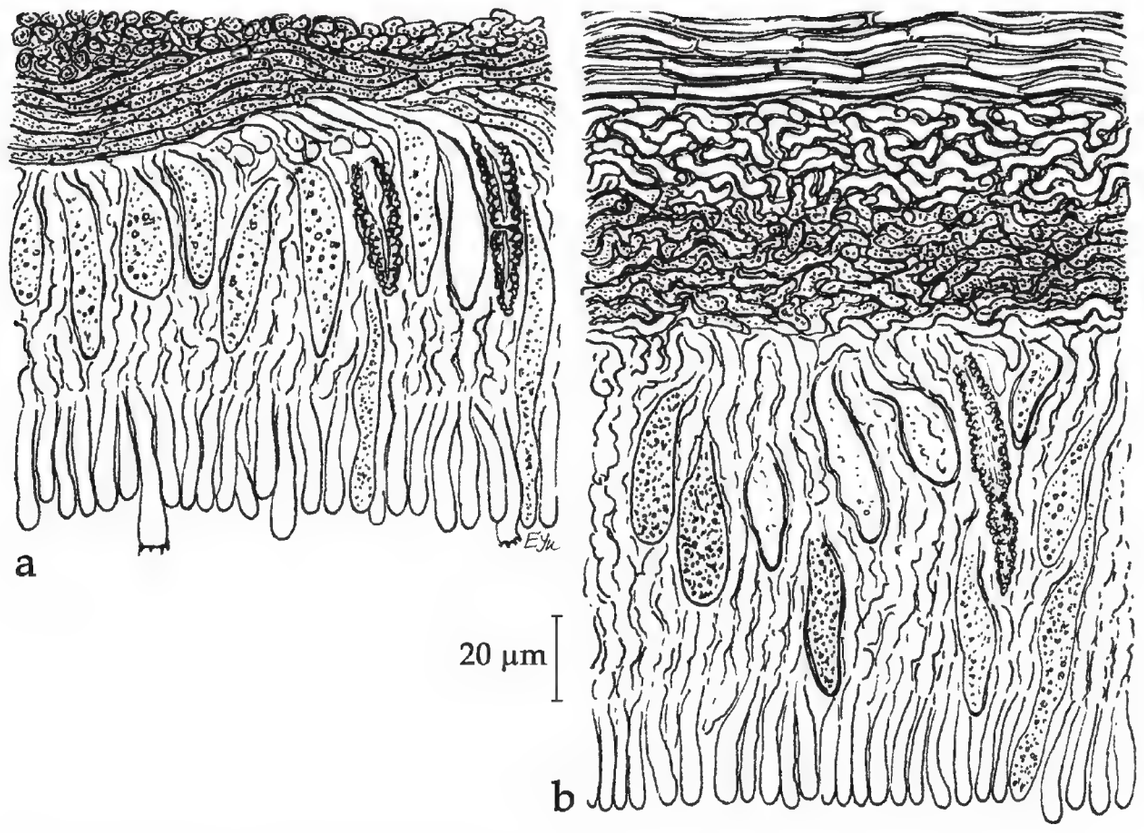

Fig. 2. Vertical basidioma sections in *Peniophora pseudonuda* (GB12298/FCUG 2384): a – in thinner part, with brown compact basal layer, b – in thicker part, with hyaline to brownish, less compact subicular hyphae.

pseudoparenchymatous tissue. The last type of subiculum occurs as tramal tissue in the teeth of the hymenophore (Fig. 4b). Subicular hyphae in *P. laeta* are usually hyaline or subhyaline, but in old basidiomata some hyphae become yellow or yellow-brown, like in *P. pseudonuda*.

We regard the differences in subiculum organization as an adaptation to subcortical or epicortical growth. In order to break and uplift the bark to expose the hymenium, the fungus develops hydnoid projections, together with thicker and looser subiculum, often containing the characteristic pseudoparenchyma. On twigs with thin bark and/or with few or no lenticels, the fungus can easily break the bark layer. However, on twigs with firm bark the fungal mycelium emerges through bark holes, apparently not being able to rupture the bark. The brown pigmentation of the epicortical subiculum in *P. pseudonuda* is considered as an adaptation to light exposure. It is well known from other *Peniophora* species that a brown subicular layer may yield a basidioma with a brownish grey or bluish grey color of the hymenium (Eriksson et al. 1978). Contrary, the basidiomata of *P. laeta* are partly covered from direct sunlight during the subcortical basidioma formation and the subicular layer consists of hyaline or subhyaline hyphae. Based on samples collected in Eurasia from Sweden to Iran, an emended morphological description of *P. laeta* has been constructed.

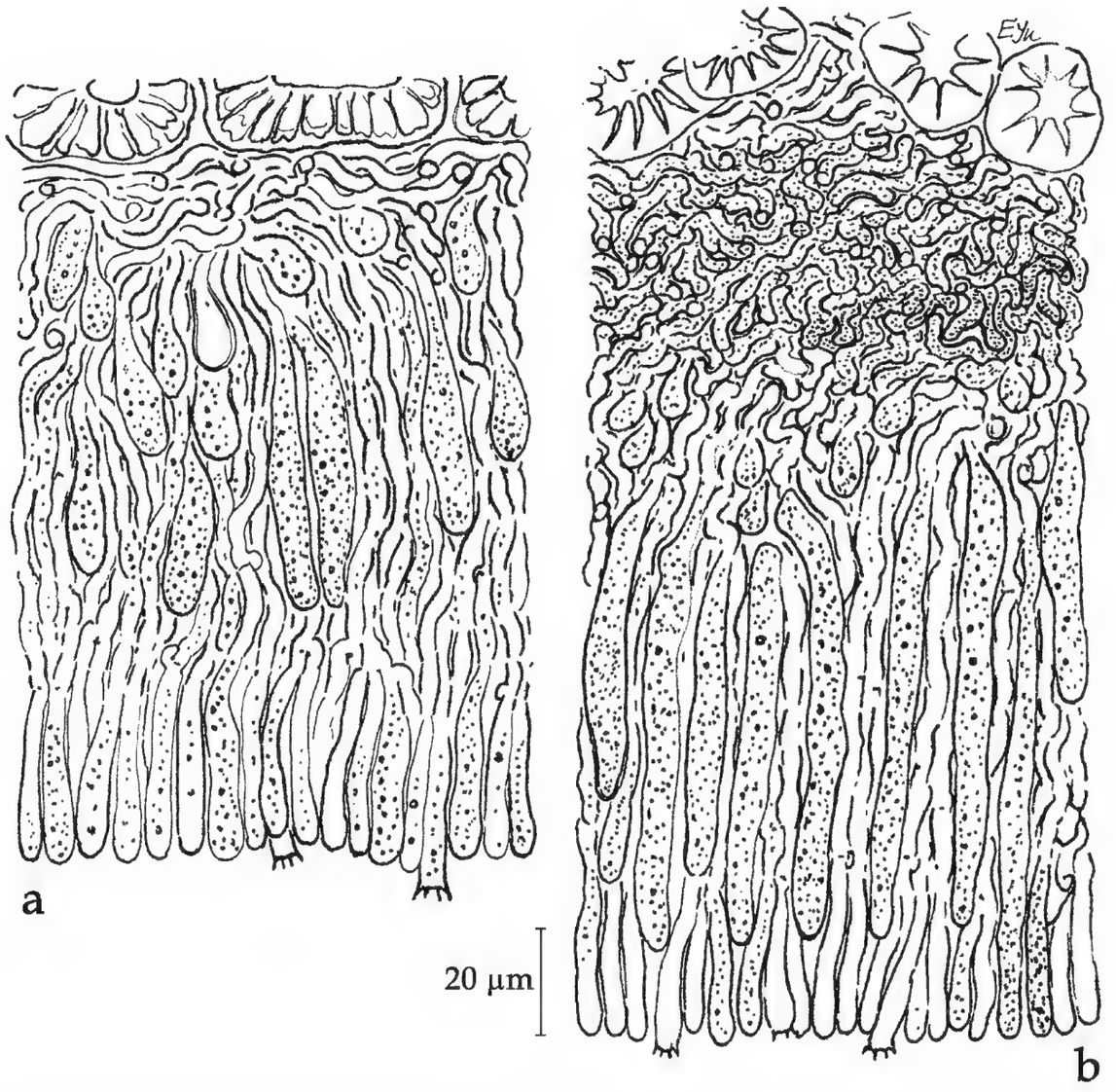

Fig. 3. Vertical basidioma sections in *Peniophora laeta* (MSK 6943): a – in thinner part, with scarce hyaline subicular hyphae, b – in thicker part, with moderately developed yellow compact subiculum and elongated gloeocystidia.

Peniophora laeta (Fr.) Donk Figs. 1–4

= *Peniophora pseudonuda* Hallenb.

Basidioma annual, resupinate, closely adnate, developing under the bark and extending through and rupturing the bark upon growth, or – alternatively – extending on bark and soon becoming confluent, ceraceous, 80–150 µm thick in smooth parts; hymenium surface pruinose under a lens, color variable – creamish, creamish-orange with reddish tint, or bluish grey; hymenophore smooth to irregularly tuberculate-odontoid, teeth scattered, up to 2.5 mm long and 1 mm wide, occasionally joined and aggregated; margin abrupt to thinning out.

Hyphal system monomitic, hyphae with clamps, arranged vertically in subhymenium, 3–4 µm wide, thin-walled, not changed in KOH. Subiculum 40–400 µm thick, almost lacking in some collections; texture variable, from dense, consisting of agglutinated golden brown hyphae, to pseudoparenchymatous in the centre of teeth, or composed of loose and intertwined, subhyaline hyphae. Cystidia of two types: 1) gloeocystidia, 40–115 × 9–20 µm, often developing deeply in the subhymenium, vesicular-clavate, becoming elongate, and reaching the hymenial surface, contents refractive, granular to homogeneous, walls thin to moderately thickened, 2) metuloids (encrusted pointed cystidia), also developing deeply in the subhymenium, rare or even lacking in some collections, crystallized part 15–37 × (7.5–)10–12 µm. A few naked and pointed cystidia are sometimes present among the basidia, only slightly projecting above the hymenium. Basidia subcylindrical to narrowly clavate, little flexuose, 35–50 × 5–6.5 µm, with a basal clamp, with four sterigmata, walls slightly thickened in mature basidia. Spores subcylindrical, slightly depressed adaxially, (7.2–)8–11.5(–13) × (2.2–)3–4.5(–5) µm, with a small apiculus, contents hyaline or subhyaline, walls smooth, thin, CB+, IKI–.

Substrata — On dead, still-attached, sometimes fallen, thin (0.2–1.5 cm) twigs and branches of hardwood trees. In Europe mostly found on *Carpinus betulus*, occasionally *Quercus robur*; in W. Asia also found on *Corylus avellana*, *Fagus orientalis*, *Parrotia*, *Quercus*. In North America it has only been recorded from *Amelanchier*, which suggests that this material needs to be re-examined.

Specimens examined — **BELARUS: Mahilyou oblast**, Asipovichy, Brytsalavichy, on *Carpinus*, 6.IX.2006, Yurchenko (MSK-F 6738; d); **Minsk oblast**, Salihorsk, Hotsk, on *Carpinus*, 20.VI.2008, Yurchenko (MSK-F 6943); Hlusk, Slaukavichy, on *Carpinus*, 1.X.2008, Yurchenko (MSK-F 7076; d); **Homel' oblast**, Petrykau, Adasi, on *Carpinus*, 19.X.1998, Yurchenko (MSK-F 4560; d). **GEORGIA:** Colchis, Kulo, alt. 1200 m, on *Corylus avellana*, 5.X.1963 Parmasto (TAA 16745; nd). **IRAN: E. Azerbaijan**, W. Kaleibar, Makidi, on *Carpinus*, 3.X.2006, Ghobad-Nejhad 413A (nd); **Golestan**, Golestan National Park, on fallen hardwood, 26.IV–8.V.1978, Hallenberg 2555 & Danesh-Pajuh (**holotype** of *Peniophora pseudonuda*, GB; nd). **ROMANIA:** Cluj near Poieni, on *Carpinus*, 23.X.1985, Hallenberg 9358 (GB-0073654; FCUG 1475; d). **RUSSIA**: **Adygeya**, Maykop, Guzeripl', on *Fagus orientalis*, 14.IX.2003, Kotiranta 22517 (HK ref. herb.; dupl. MG ref. herb.; nd); Krasnodar, Mostovskoj, Psebaj, on fallen hardwood, 15.IX.1991, Hallenberg 12298 (GB-0073645; FCUG 2384; nd); Stavropol', Kislovodsk, on *Carpinus*, 20.VIII.2000, Yurchenko (MSK-F 6688; nd). **SWEDEN: Gotland**, Visby, DBW Botanical Garden, on *Carpinus betulus*, 5.X.1984, Nordin 9428 (H; d); Scania, Stenshuvud, on *Carpinus*, 1.X.1984, Hallenberg 8557 (GB-0073663; FCUG 1266; d). **UKRAINE: Kyiv oblast**, Rzhyshchiv, Hrebeni, on *Carpinus*, 8.IX.1973, Soldatova (KW 17598, dup. in MSK; d); **Kirovhrad oblast**, Holovaniv, on *Carpinus*, 24.VIII.1973, Soldatova (KW 17590; dup. in MSK; d); **Cherkasy oblast**, Kaniv Reserve, on *Quercus robur* (!), 10.IX.2003, Akulov (CWU myc Ch-24; d); **Crimea**, Sudak, Lesnoe, 2.VIII.2001, Yurchenko (MSK-F 5981; d).

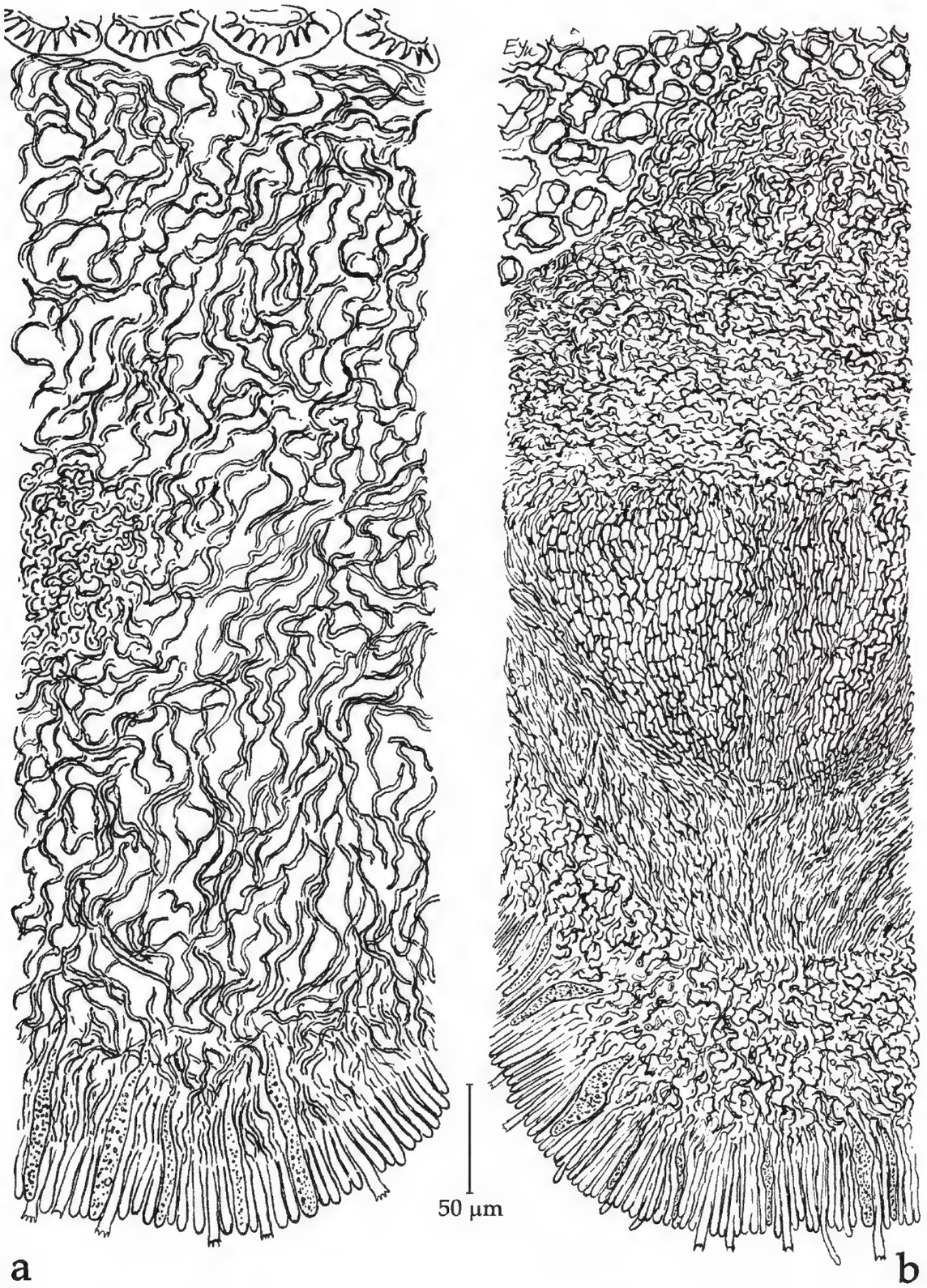

FIG. 4. Vertical basidioma sections in *Peniophora laeta* (MSK 6943) in thicker part and hymenophore projections: a – a portion with subiculum of loose hyaline hyphae, b – a portion with hyaline to yellowish subiculum, with pseudoparenchymatic insertion (center).

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